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CHROMOSOME REDUCTION IN THE MICROSPOROCYTES OF LILIUM TIGRINUM.[1]

JOHN H. SCHAFFNER.

(WITH PLATES XII AND XIII)

THE progress recently made in our knowledge of hybrids has given a new impetus to the study of chromosome reduction. Unfortunately, there is still much disagreement in the accounts of various observers. In order to continue my own investigations on a very favorable object, the microsporocytes of *Lilium tigrinum* were selected, since material is easily obtained in large quantities. CHAMBERLAIN has studied the pollen grain of this plant and has also given figures of the microsporocyte in the spirem stage. The chromatin granules are exceptionally distinct, and this facilitates the correct interpretation of the complex figures to be seen in the reduction karyokinesis.

Recently papers on the reduction division have been published by FARMER and MOORE, STRASBURGER, MONTGOMERY, WALLACE, and others, which are in essential agreement with the interpretations of DIXON on *Lilium longiflorum* and my own observations on *Lilium Philadelphicum* and Erythronium. On the other hand BERGHS, GRÉGOIRE, and ALLEN have come to somewhat different conclusions. The observations of ROSENBERG on Drosera have opened up an important field of investigation on the individuality of the chromosome. These papers have been reviewed so recently by various writers that it is needless to discuss the results here. It is sufficient to say that it must appear to an impartial judge that the cytologist is at present able to see in his preparations almost anything which may be conceived of as taking place in the structures investigated. This, however, should not hinder work in such an important field, for the proper interpretation can be attained only by continued observation. Little can be regarded as certain until there is a more general agreement among competent investigators. So far as the present research is concerned, the extent and variety of the preparations studied seem to preclude the possibility of mistake. Such doubts as were expressed in my former papers have been practically removed. So far as the writer is concerned, the interpretation given below is conclusive. Another investigator might perhaps come to different conclusions by using other methods.

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[1] Contributions from the Botanical Laboratory of Ohio State University. XXIV.

MATERIALS AND METHODS.

Stamens of various ages were collected in Clay County, Kansas, during July 1904; killed in weak chrom-acetic acid (chromic acid 0.3^{gm}, glacial acetic acid 0.7^{cc}, water 99^{cc}), passed gradually through the grades and preserved in 70 per cent. alcohol, imbedded in paraffin, cut 10–18 μ thick; and stained on the slide. Some old slides, the material of which had been killed in the ordinary chrom-acetic acid solution, were also at hand. Various stains and combinations were used, but for bringing out the chromatin network and chromatin granules of the early stages, Delafield's haematoxylin, when properly developed, gave by far the best results, being superior in this respect to either Heidenhain's iron-alum-haematoxylin or safranin and gentian violet. Nucleoli, both in the nuclear cavity and in the cytoplasm, are stained very distinctly by the safranin-gentian-violet combination, but are only slightly affected by Delafield's haematoxylin.

I am indebted to my wife, MABEL SCHAFFNER, for the preparation of most of the two hundred serial slides on which the present paper is based.

INVESTIGATION.

Before the microsporocytes are beginning to separate the prominent chromatin network is being transformed into slender delicate threads. These threads appear to be discontinuous in some places. However, the appearance may be due to injury during the process of cutting. The threads are small in diameter as compared with the single chain of spherical chromatin granules (*figs. 1–3*). After the spirem becomes fully developed it shows no free ends and is much wound, looped, and twisted. In this stage it appears to be entirely free in the nuclear cavity and is usually in the so-called synapsis stage. Sometimes the contraction is to one side of the nuclear cavity, sometimes near the center, but often little or no contraction is evident. Whether this is an artifact or a real stage in the process of karyokinesis appears still to be an open question. Such contractions are so easily produced by the ordinary killing reagents used, and have been described for such a variety of the early stages of nuclear division, that it seems to me no importance is to be attached to observations which have been made on killed material. So far as any opinion is to be expressed upon the appearances in *Lilium tigrinum*, I am still convinced that the extraordinary distortions commonly figured owe their origin to the action of the fluids used before imbedding in paraffin.

The spirem remains simple during the entire synapsis. The linin thread becomes thickened and the chromatin granules are usually more or less elongated (*figs. 4, 5*). At this stage the spirem has already a strong tendency to be thrown into loops and coils (*fig. 4*). After the microsporocytes have become partly separated and more spherical, they were rarely observed to be in synapsis. The nuclear cavity enlarges and the chromatin ribbon becomes thicker, with the granules still more prominent (*figs. 6–8*). This is an important point to consider, for we have here a clear case of sporocytes, long past the supposed synapsis stage, showing with remarkable clearness a simple continuous spirem, with a single chain of chromatin granules.

Synapsis, therefore, can have nothing to do in this case with a supposed longitudinal conjugation of two spirems or two networks of chromatin before the spirem is formed. After the nuclei have passed on to the stage represented in *fig. 6*, the stages are so easily followed and the threads so prominent, that a longitudinal conjugation, if one occurred, could not escape notice. Shortly after the stage shown in *fig. 6* the spirem begins to show double rows of elongated chromatin granules, but the relative quantity of chromatin ribbon present in the nuclear cavity is not much diminished (*figs. 9, 10*). If the amount of spirem were diminished one-half by a longitudinal conjugation the fact would certainly be noticeable. Often a part of the spirem appears still single, or it will appear with a double row of granules and gradually change to a row apparently single (*figs. 11–17*). The appearance would be the same whether the granules were dividing or conjugating. The uniformity of the pairs of granules on the linin thread is remarkable, and the pairs themselves suggest a division. If a conjugation of the chromatin granules were established, it would certainly show an almost inconceivable regularity. The granules would have to be definite and fixed, of the same number in both the egg and sperm, and not be subject to increase or diminution (*figs. 14–16*). In the early stages of the spirem there are, of course, numerous instances of threads lying parallel, side by side, but these appearances are equally common after the chromatin granules appear double (*figs. 15, 17*).

The ribbon now begins to show an arrangement into definite loops (*fig. 10*). It becomes much shorter and thicker (*figs. 18, 19*) and finally shows a definite twisting together into twelve loops, which have their heads toward the nuclear wall (*fig. 20*). At this stage the chromatin granules can still be distinguished lying side by side (*fig. 21*). But at this time the whole ribbon begins to undergo a change, so that it stains of a uniform, dense color throughout, and before the loops separate all evidence of chromatin granules is lost (*figs. 22, 23*). That the loops shown in *figs. 18–23* are the incipient chromosomes is self-evident. By no manner of interpretation can such a conclusion be explained away. By the time the twelve loops have separated, the nucleoli have entirely disappeared from the nuclear cavity (*fig. 24*). The nucleoli break up into micronucleoli and are thrown out into the cytoplasm (*figs. 38, 51*). The figures in which they do not appear were taken from material stained in such a way that the nucleoli were not evident or not very distinct.

The chromosomes are exceedingly interesting on account of the many fantastic shapes produced by the coiling of the ribbon. A series of distinct shapes is given in *figs. 25–37*. Occasionally the loop shows evidence of its double nature (*fig. 29*), but usually it appears homogeneous throughout. Much time was spent in a study of these chromosomes, and the variety of shape and coil could be extended indefinitely. In all cases the chromosome is continuous, the outer end of the loop always being closed. Occasionally the coiling takes place in such a way as to form a double loop (*figs. 26, 27, 35*). The chromosome is situated on the spindle with its head or closed end outward (*figs. 28–30*). Sometimes it is very difficult to unravel the nature of the coil, as in *fig. 35*. In other chromosomes it is an easy matter to follow out the details of the loop, as in *fig. 28*.

The spindle threads are evidently attached some distance back of the free limbs of the loop (*figs. 25–28*). The limbs are gradually uncoiled and pulled apart. They are separated at the closed outer end. A transverse splitting of the chromosome is thus accomplished. There is no evidence that the two limbs of the chromosome are a male and female chromosome joined end to end and twisted together until they make a longitudinal pair. But theoretically such a proposition is easily possible or even probable. Because the spindle threads are attached some distance from the ends of the limbs, the daughter chromosomes are developed as V- or U-shaped loops (*figs. 40, 43, 51*). In favorably stained sections some evidence of chromatin granules may be observed. These still show a distinct pairing in some cases (*fig. 44*), but in others the arrangement is considerably disturbed (*fig. 45*). The daughter chromosomes, as they appear in the daughter star, are of various forms and are sometimes twisted (*figs. 44–50*).

The micronucleoli are gradually collected below the two daughter skeins, and are finally all inclosed in the nuclear cavities of the daughter nuclei (*figs. 52, 53*). The daughter chromosomes do not appear to form a very definite resting network, but are transformed into the mother skein of the second division rather rapidly. Whether a continuous ribbon is formed was not ascertained. The loops are already separate at an early stage, and it is possible that the daughter chromosomes of the first division, after forming an imperfect reticulum, break up directly into the twelve chromosomes of the second division (*fig. 54*). This point, however, is doubtful. But the absence of a definite resting stage in sections having microsporocytes with loose daughter skeins in close proximity to loose mother skeins of the second division, gives support to the above supposition. The micronucleoli are again distributed in the cytoplasm before the mother-star stage of the second division (*figs. 54, 55*). The karyokinetic figures of the second division are easily distinguishable from those of the first. This is especially true of the mother star (*figs. 38, 55*). The chromosomes in the second division have their free ends directed outwards, as in an ordinary vegetative division. Commonly they are more or less tangential to the spindle. Sometimes, however, they stand at right angles, as represented in *figs. 56, 57*.

One of the most difficult points to determine was the nature of the splitting in the second division. However, it was definitely ascertained that the splitting is longitudinal. Dividing chromosomes are represented in *figs. 58–61*. The daughter loops are completely separated very early in their migration towards the poles (*fig. 62*). They are also of various shapes. Frequently straight chromosomes are present in the daughter star and very commonly the J shape predominates (*figs. 63, 64*). U and V shapes are also present. After the chromosomes have passed into the daughter skein stage they show an irregular outline with a row of irregular chromatin granules (*figs. 65, 66*). The chromosomes develop into irregular networks, showing the remains of the original loops, and thus pass into the spore tetrad stage (*fig. 67*).

During the early germination stages of the microspores the figures are again remarkable for the large nucleoli in the cytoplasm (*figs. 68–70*). From the appearance of the nuclei during the several divisions, it is evident that the nucleoli do not contribute directly to the formation of the chromosomes, but that they are uniformly thrown out into the cytoplasm, in a fragmented condition, during the earlier stages of karyokinesis.

No study of the achromatic structures was attempted, and though some interesting points were observed from time to time, there was nothing which calls for special mention.

SUMMARY.

1. The first division of the microsporocyte is a true reduction division.
2. A continuous spirem is formed with a single row of chromatin granules.
3. The spirem passes through and comes out of synapsis without a conjugation or division of chromatin granules.
4. The chromatin granules divide but the linin thread does not show a distinct separation.
5. The continuous spirem shortens and thickens and twists up into twelve loops, which are the incepts of the twelve separate chromosomes.
6. The chromosomes are arranged in the mother star with the loop or head end turned outwards and the spindle threads are attached near the ends of the free limbs or about half way between the free ends and the head.

7. During the metakinesis stage the chromosomes uncoil and separate by a transverse division at the middle.

8. The chromosomes of the second division appear to represent the daughter chromosomes of the first division.

9. The division of the chromosomes in the second nuclear division is longitudinal.

10. The nucleoli fragment and pass out into the cytoplasm during the first and second divisions and also during the germination of the microspore.

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EXPLANATION OF PLATES XII AND XIII.

The plates are reduced five-eighths in reproduction. The figures representing entire cells and nuclei were studied with a Leitz no. 4 ocular and $\frac{1}{16}$ oil immersion objective; the others with a Zeiss no. 12 ocular and a Leitz $\frac{1}{16}$ oil immersion objective.

PLATE XII.

FIG. 1. Microsporocyte with chromatin network beginning to form the spirem.

FIG. 2. Microsporocyte with delicate threads showing a single row of prominent chromatin granules.

FIG. 3. Single thread of the same.

FIG. 4. Sporocytes beginning to separate. Nucleus with distinct continuous, contracted spirem in the so-called synapsis stage.

FIG. 5. Single thread of the same showing a single row of chromatin granules.

FIG. 6. Sporocyte some time after the synapsis stage, showing prominent spirem with single row of chromatin granules. The free ends are cut.

FIGS. 7, 8. Single threads of the same.

FIG. 9. Sporocyte with chromatin granules mostly double and flattened in appearance.

FIG. 10. Somewhat later stage, showing the beginning of looping of spirem, and division of chromatin granules. One nucleolus beyond the nuclear cavity.

FIGS. 11–17. Single threads from the same stage, showing chromatin granules still single, some with granules partly double, and others with typical double rows of granules.

FIG. 18. Microsporocyte with loops nearly developed.

FIG. 19. A single loop of the same stage.

FIG. 20. Chromatin loops some time before separation; somewhat cut.

FIG. 21. A single loop from the same stage.

FIG. 22. Section of microsporocyte, showing chromatin loops near the time of separation.

FIG. 23. A single loop from the same nucleus.

FIG. 24. Microsporocyte, showing the twelve chromosomes.

FIGS. 25–37. Individual chromosomes, showing various types of loops and coils and their position on the spindle threads.

PLATE XIII.

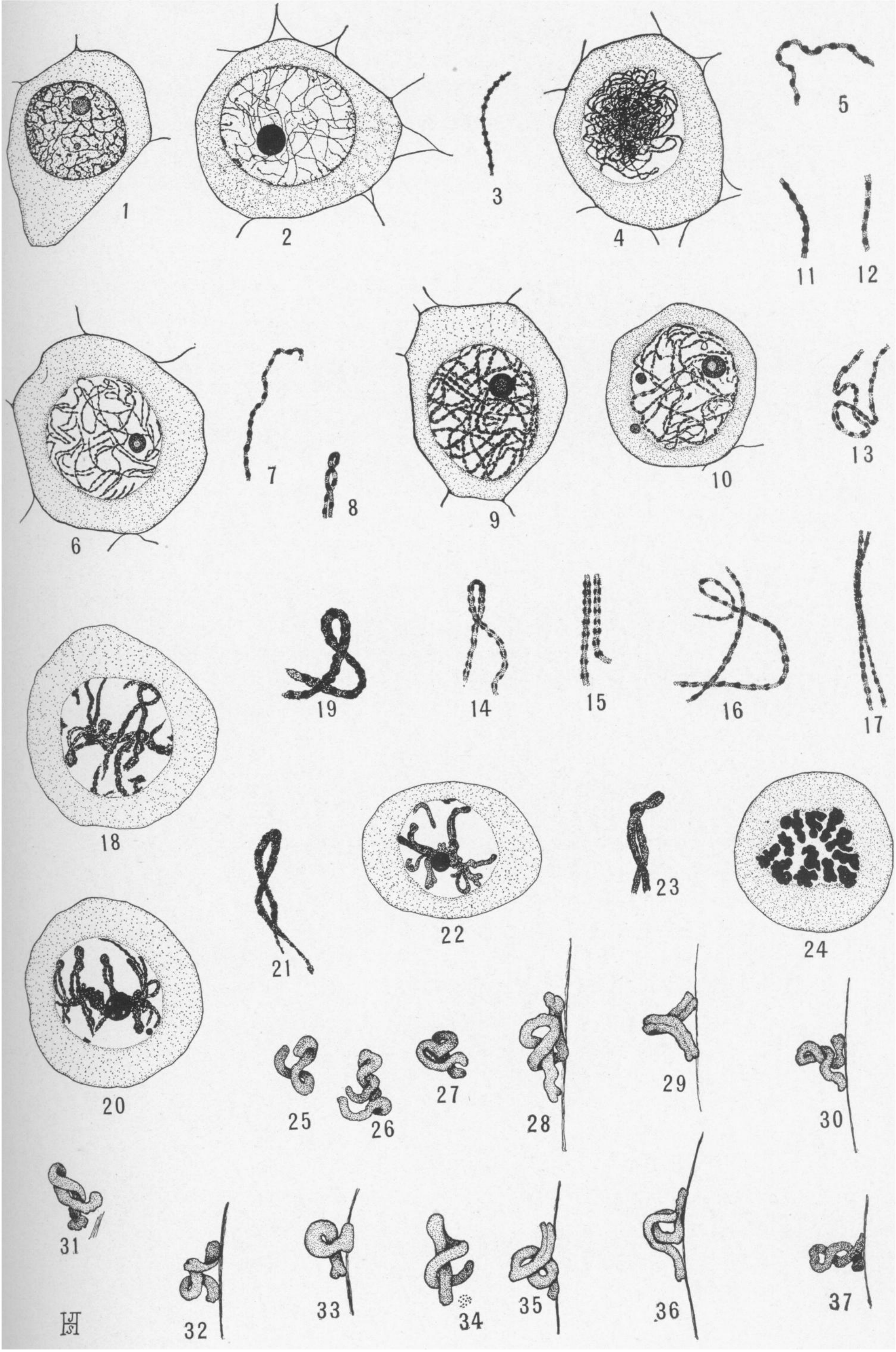
FIG. 38. Mother star, showing position of chromosomes. Micronucleoli in the cytoplasm.

FIGS. 39–42. Chromosomes from mother-star stage, showing mode of separation of limbs of loops.

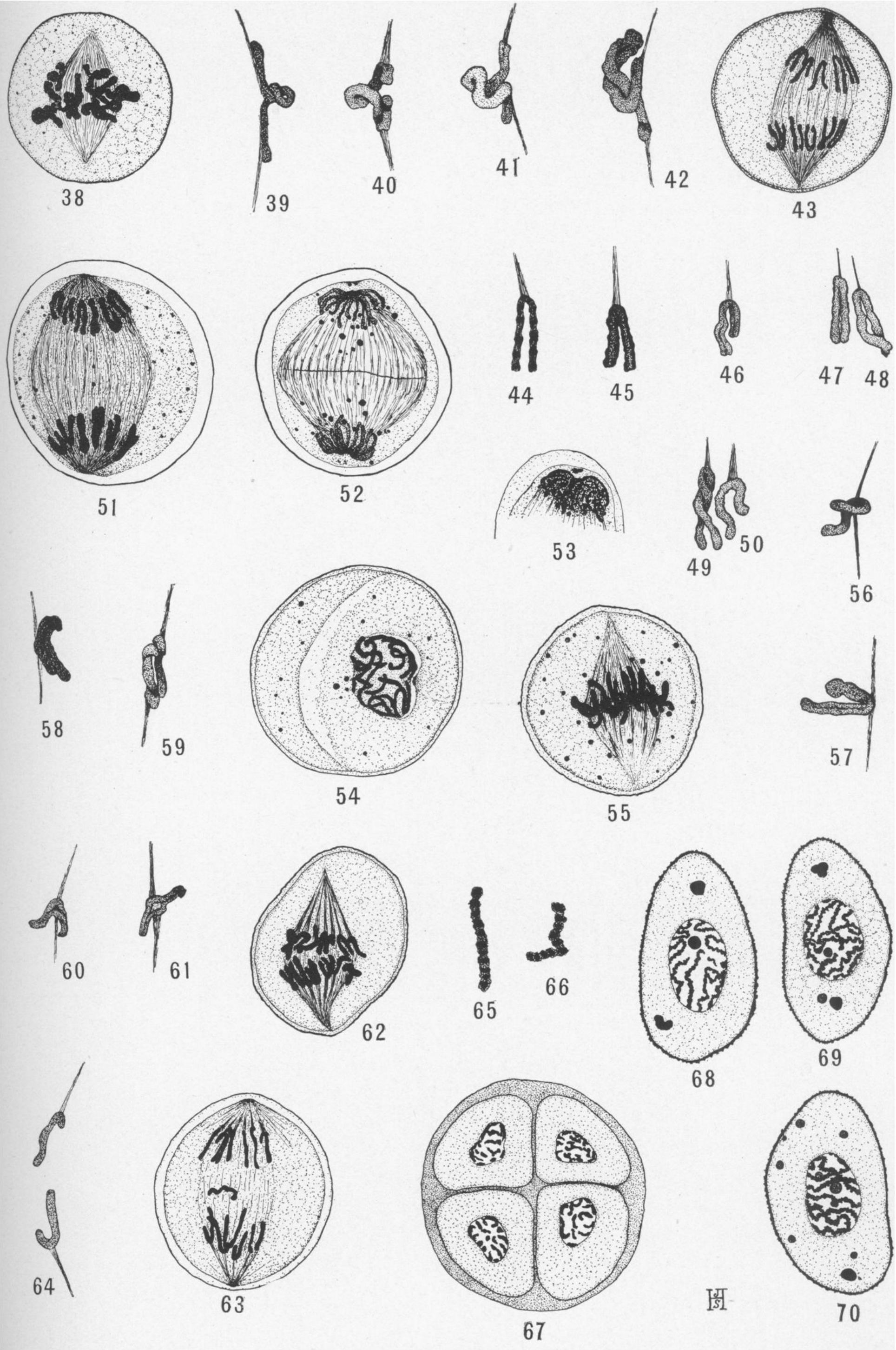
FIG. 43. Microsporocyte with daughter stars.

FIGS. 44–50. Daughter chromosomes from the daughter star, showing character of of the loops.

FIG. 51. Daughter-star stage, with micronucleoli in the cytoplasm.



SCHAFFNER on LILIUM



SCHAFFNER on LILIUM

FIG. 52. Loose daughter-skein stage with micronucleoli collected below the open ends of the chromosomes.

FIG. 53. End of loose daughter-skein stage, with micronucleoli collected among the chromosomes.

FIG. 54. Beginning of second division, with chromatin loops in the nucleus and micronucleoli in the cytoplasm.

FIG. 55. Mother star of the second division, showing characteristic appearance of the chromosomes, with micronucleoli in the cytoplasm.

FIGS. 56, 57. Single chromosomes from the mother-star stage, showing position on the spindle threads.

FIGS. 58–61. Chromosomes from the metakinesis stage, showing the nature of the longitudinal splitting.

FIG. 62. End of metakinesis stage.

FIG. 63. Daughter-star stage of second division.

FIG. 64. A pair of daughter chromosomes from the daughter-star stage of the second division.

FIGS. 65, 66. Pieces of chromosomes at the end of the second division, showing a single row of irregular chromatin granules.

FIG. 67. Tetrad at end of second division.

FIG. 68. Microspore at beginning of germination, with two large nucleoli in the cytoplasm.

FIG. 69. Same stage as the preceding, showing three nucleoli in the cytoplasm.

FIG. 70. Microspore in germination stage, showing a number of nucleoli in the cytoplasm.